Characterization of Minor 2'-Deoxyguanosine-PhIP Adducts Formed *in Vitro* by Reaction of N^2-acetoxy-PhIP with DNA

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PhIP minor adduct products, as well as previously-characterized dG-C8-PhIP, were identified from the reaction of *N*-acetoxy-PhIP with macromolecular DNA or 2'-deoxyguanosine (dG). Macromolecular DNA adducts were characterized by absorption and fluorescence spectroscopy as well as ^{32}P-postlabeling, while the nucleosidic adducts were characterized by UV/vis, fluorescence, mass spectrometry, and in some cases proton NMR spectroscopy. Macromolecular PhIP adduct formation yielded primarily adducts to the C8 atom of guanine (~ 70%) plus several additional minor adducts. These additional minor DNA adducts were more polar than, and formed over time from, dG-C8-PhIP. Adduct formation from *N*-acetoxy-PhIP reaction with dG yielded mainly dG-C8-PhIP (~ 80 - 90 %, molecular weight = 489 Da) and at least three additional polar adducts, all with molecular weight of 507 Da. Results from collisional dissociation of these molecular ions confirmed PhIP-related adducts and suggest ring-opened species. Incubation of dG-C8-PhIP under alkaline conditions (pH 12.5) indicated oxidation, yielding a single adduct (MW = 505 Da) not produced in the reaction of dG with N-acetoxy-PhIP. Finally, the ^{32}P-postlabeling data suggested that some or all of the minor PhIP adducts are formed to DNA *in vivo*, although their significance is as yet unknown. *Work performed under the auspices of U.S.D.O.E. by Lawrence Livermore National Laboratory under contract W-7405-ENG-48, and supported by NIH grant CA55861.*